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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Group Art Unit: 1651

Examiner: Meller, M.

Confirmation No.: 4119

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7/1/03

In re Patent Application of

HAMET, P. et al.

Application No.: 09/480,260

Filed: January 11, 2000

For: PRE-CONDITIONING AGAINST CELL DEATH

DECLARATION

I, Stanley H. Appel, MD, Professor of Neurology, hereby declare that:

1. I am Professor and Chairman of the Department of Neurology, Director of the MDA/ALS Research and Clinical Centre, and Director of the Alzheimer's Disease Research Centre at Baylor College of Medicine, Houston, USA. I have authored or co-authored over 300 published research papers in the field of neurology, over the past 45 years. A copy of my Curriculum Vitae (omitting the list of publications) is attached hereto.
2. I have read US Patent No. 5,834,030 Bolton (hereinafter "the Bolton patent") and the subject US Patent Application Serial No. 09/480,260.
3. The Bolton patent teaches that a process of contacting autologous blood with ozone gas and ultraviolet radiation *ex vivo*, and administering the treated blood to the patient from whom it originated, has the effect of increasing the nitric oxide concentration in the patient's blood. It proceeds to suggest that the process therefore provides a potential treatment for disease states presently believed to be associated with

inadequate nitric oxide levels in the blood. Such states include "high blood pressure; neurological conditions such as depression; tumors; bacterial, viral, protozoal, and fungal infections, and impotence" (column 6, lines 1-3). The Bolton patent postulates that, in the brain, nitric oxide mediates the actions of the excitatory neurotransmitter glutamate in stimulating cyclic GMP concentrations, to exert possible beneficial effects on depression.

4. No expert would classify depression as a neurodegenerative disease. Depression is not associated with apoptosis, to my knowledge, by experts in this field. Nothing in the Bolton patent teaches that depression is associated with apoptosis.
5. Neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, in contrast to depression, are related to excessive amounts of brain cell death (neurotoxicity) as a result of excessive apoptosis and/or necrosis, and inflammation, resulting largely from the development or activity of reactive oxygen species in the brain cells. Nitric oxide is neurotoxic.
6. The neurotoxicity of nitric oxide has been extensively studied. Nitric oxide exerts its neurotoxic effects via several mechanisms. Nitric oxide has characteristics of a free radical and can combine in the cell with superoxide anions to form peroxynitrite, a highly destructive radical moiety. The resultant reactive oxygen species can induce significant oxidative stress that causes lipid peroxidation and produces functional alterations in proteins and DNA, eventually leading to neuronal cell death.
7. A process taught to increase the production of nitric oxide would be regarded as totally inappropriate for treatment of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, which involve excessive neuronal cell death.
8. In Parkinson's disease, excessive release of oxygen free radicals during enzymatic dopamine breakdown, impairment of mitochondrial function, production of

inflammatory mediators, loss of trophic support and apoptosis leading to excessive neuronal cell death are believed to be important mechanisms in the disease.

9. Alzheimer's disease is characterized by amyloid plaques, neurofibrillary tangles, gliosis, and neuronal and synaptic loss. The underlying pathological dysfunction is believed to involve the toxic effects of beta amyloid, possible tau induced pathology, inflammation, oxidation, excitotoxicity and apoptosis.
10. The main mechanisms common to neurodegenerative diseases namely increased oxidative stress, altered mitochondria, enhanced inflammatory processes, increased glutamate excitotoxicity, protein aggregation and altered proteosomal function, and altered calcium homeostasis, are not pertinent to depression, and are not mentioned in the Bolton patent.
11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

EXECUTED at Houston, Texas, this 10 day of June, 2003.



Stanley H. Appel.

CURRICULUM VITAE

NAME: Stanley Hersh Appel

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Baylor College of Medicine
Houston, Texas 77030
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DATE AND PLACE OF BIRTH: May 8, 1933; Boston, Massachusetts

SOCIAL SECURITY NUMBER: 033-24-3034

EDUCATION: Harvard College, A.B. degree, 1954
Columbia College of Physicians and
Surgeons, M.D. degree, 1960

PROFESSIONAL BACKGROUND:

1977- Professor and Chairman, Department of Neurology; Director, Jerry Lewis Neuromuscular Disease Research Center; Director, Vicki Appel MDA/ALS Center; and Director, Alzheimer's Disease Research Center, Baylor College of Medicine, Houston, Texas

1977- Professor, Department of Biochemistry, Neurosciences, and Molecular Physiology; Baylor College of Medicine, Houston, Texas

1976-1977 James B. Duke Professor of Medicine (Neurology), Duke University Medical Center, Durham, North Carolina

1971-1976 Professor of Medicine (Neurology), Duke University Medical Center

1969-1977 Chief, Division of Neurology, Duke University Medical Center

1967-1971 Associate Professor of Medicine (Neurology), Duke University Medical Center

1967-1977 Associate Professor of Biochemistry, Duke University Medical Center

1966-1967 Assistant Professor of Medicine and Neurology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

1965-1966 Chief Resident in Neurology; Associate in Medicine and Neurology, University of Pennsylvania School of Medicine

1964-1965 Associate in Medicine (Neurology), Duke University Medical Center

1963 July and August, Visiting Scientist in Genetics and Neurology, Jackson Laboratory, Bar Harbor, Maine

1962-1964 Research Associate, Laboratory of Molecular Biology, National Institutes of Health, Bethesda, Maryland

1961-1962 Resident in Neurology, Mount Sinai Hospital, New York, New York

1960-1961 Intern in Medicine, Massachusetts General Hospital, Boston, Massachusetts

HONORS:

Alpha Omega Alpha
Research Career Development Award, 1965-1973
Certified, American Board of Psychiatry and Neurology, 1968
Research Award, Society of Biological Psychiatry, 1969

Golden Apple Award, Outstanding Teacher, Duke University School of Medicine, 1970; nominated 1977
G. Milton Shy Visiting Professor, Columbia College of Physicians and Surgeons, 1971
Senior Fellow, Center for the Study of Aging and Human Development, Duke University Medical Center, 1975-1977
James B. Duke Professor of Medicine (Neurology), 1976-1977
Favored Faculty, Baylor College of Medicine, 1979
John F. Sullivan Visiting Professor, Tufts-New England Medical Center, 1980
Outstanding Faculty, Baylor College of Medicine, 1982
Francis McNaughton Lecture, Montreal, Canada, 1984
National Vice President, Muscular Dystrophy Association, 1989
Faculty Associate, Huffington Center on Aging, 1995
P&S Alumni Gold Medal for Excellence in Biomedical Research, 1997 College of Physicians & Surgeons, Columbia University, 1997
Cal Ripken/Lou Gehrig Visiting Professor, Johns Hopkins University, 1998
Morris Bender Visiting Professor, Department of Neurology, Mount Sinai School of Medicine, 1999
Lou Gehrig Memorial Lecture, College of Physicians & Surgeons of Columbia University, 1999
Sheila Essey Award For ALS Research - American Academy of Neurology

PROFESSIONAL COMMITTEES AND SOCIETIES:

Alzheimer's Disease and Related Disorders Association
Medical and Scientific Board, 1980-1990
Alzheimer's Disease and related Disorders Association
Advisory Board, 1990-1992
American Academy of Neurology
Chairman, Section on Neurochemistry, 1973-1975
Program Committee, 1973-1975
American Committee for the Weizmann Institute of Science, Houston Region
Scientific Advisory Board, 1994-
American Neurological Association
Councilor, 1978-1982
American Society of Biological Chemists
American Society of Clinical Investigation
American Society of Neurochemistry
Council, 1973-1977
Chairman, Program Committee, 1973-1974
Amyotrophic Lateral Sclerosis Society of America
Scientific Advisory Committee, 1975-1980
Chairman, 1980-1984
Amyotrophic Lateral Sclerosis Association
Co-Chairman, Scientific Advisory Board, 1985-1987
The British Neurological Research Trust
Member, Scientific Board, 1988-1990
Experimental Gerontology
Associate Editor, 1985-1989

International Society for Neurochemistry
Clinical Committee, 1975-1977
Multiple Sclerosis Medical Advisory Committee, Southeast Texas--*ex officio* Member
Muscular Dystrophy Association,
National Headquarters, Scientific Advisory Committee, 1994
Myasthenia Gravis Foundation, Inc.
Medical Advisory Board
National Huntington's Disease Association
National Science Council, Chairman, 1976-1981
National Institutes of Health, Division of Research Grants
Neurology A Study Section, 1970-1974
NINCDS Studies on Regeneration, 1974-1975
Neurology B Study Section, 1978-1979
Neurology A Study Section, 1979-1981
Chairman
Society for Neuroscience
Councilor, 1978-1982
Stroke Group of Houston
Board of Advisors, 1985-1990
USC Alzheimer's Disease Research Center
External Review Committee, 1990-1992
Veterans Affairs Research and Career Development Study
Section, 1971-1975

EDITORIAL BOARDS:

Neurology, 1972-1976
Journal of Neurochemistry, 1976-1981
Experimental Neurology, 1977-1991
Muscle and Nerve, 1977-1987
Annals of Neurology, 1978-1982
Developmental Neuropsychology, 1993-1995
Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders, 1999-

ASSOCIATE EDITOR:

Neurochemistry Research, 1976-1979
Journal of the Neurological Sciences, 1983-1986
Experimental Gerontology, 1984-1989

EDITOR-IN-CHIEF

Neurology Clinics - Ciba Geigy, 1980-1982
Current Neurology, Vol. 3, 1981-1997

RESEARCH AND CLINICAL INTERESTS:

Membrane and receptor biochemistry; molecular biology of the nervous system, neuroinflammation in ALS, Parkinson's, Alzheimer's disease.

BOOKS

Stanley H. Appel

1. Goldensohn, E.S. and Appel, S.H., Editors. SCIENTIFIC APPROACHES TO CLINICAL NEUROLOGY, 1st Edition. Lea & Febiger, 1977.
2. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 3. John Wiley & Sons, 1981.
3. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 4. John Wiley & Sons, 1982.
4. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 5. John Wiley & Sons, 1984.
5. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 6. Yearbook Medical Publishers, 1986.
6. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 7. Yearbook Medical Publishers, 1987.
7. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 8. Yearbook Medical Publishers, 1988.
8. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 9. Yearbook Medical Publishers, 1989.
9. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 10. Yearbook Medical Publishers, 1990.
10. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 11. Yearbook Medical Publishers, 1991.
11. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 12. Yearbook Medical Publishers, 1992.
12. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 13. Yearbook Medical Publishers, 1993.
13. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 14. Mosby-Yearbook Medical Publishers, 1994.
14. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 15. Mosby-Yearbook Medical Publishers, 1995.
15. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 16. Mosby-Yearbook Medical Publishers, 1996.
16. Appel, S.H., Editor. CURRENT NEUROLOGY, Volume 17. IOS Press Publishers, 1997.